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Emergence of a Lyotropic Lamellar Phase: Surfactant-Aqueous Phase Contact Experiments Examined with a Cryo-Transmission Electron Microscope

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A phase penetration experiment has been conducted, employing a cryo-transmission electron microscope (cryo-TEM). With this technique, the phase transitions and the molecular rearrangement that result from the phase penetration can be studied on almost the molecular level. The technique has been applied to the emergence of a lyotropic lamellar liquid-crystalline phase, when dodecylbenzenesulfonic acid (HDoBS) is brought into contact with water or with an aqueous sodium hydroxide solution. Both phases are nebulized onto the grid. As a result, fingerprint patterns are observed by cryo-TEM, that emerge when the aqueous phase penetrates into a thin layer of surfactant. The pattern consists of a two-dimensional lamellar alignment of the surfactant molecules. In thicker parts of the surfactant layer, the penetration results in smaller units of molecular alignment.

Introduction

If we were to ask amphiphilic molecules personally how they undergo their transition into a lyotropic lamellar phase, they would have an interesting story to tell. The lamellar phase is quite a common liquid-crystalline state for amphiphilic molecules. Particularly the lamellar arrangement of amphiphiles in aqueous media has been studied frequently, both as a continuous phase and as aggregates with a lamellar arrangement of the amphiphiles.¹ We were interested how these amphiphiles are being aligned in their lamellar arrangement, starting from the undissolved or undiluted compounds. Our previous studies were concerned with one of the industrially most popular surfactants, sodium dodecylbenzenesulfonate (NaDoBS), and a mixture of NaDoBS and a nonionic surfactant of the poly(ethylene oxide) alkyl monoether type.^{2,3} In diluted aqueous solutions these surfactants form micelles. Addition of salts induces the formation of lamellar aggregates. A detailed study of this process has provided a notion of how this lamellar arrangement of molecules originates in dilute aqueous surroundings. The lamellar arrangement of amphiphiles can also emerge when water penetrates into a bulk surfactant phase, if the surfactant is prone to form a lamellar phase. A distinction can be made between a bulk surfactant phase consisting of solid, crystalline material, where the amphiphilic molecules already have a particular order in the crystal lattice, and a liquid surfactant (for instance many nonionics and dodecylbenzenesulfonic acid), where the molecules are oriented randomly in the bulk. In the latter case, the molecules must be aligned as a result of the penetration of water molecules, before they can form lyotropic liquid-crystalline phases.

These processes receive industrial interest, since the processing of many liquid household products, such as laundry and dishwashing detergents, personal products, etc., involves mixing of bulk surfactants with water and other necessary ingredients.

This process may be assessed by a so-called contact or phase penetration experiment, where amphiphilic molecules are brought into contact with water or an aqueous phase. The interpenetration is usually followed by light microscopy. This type of experiment has proven to be fruitful and is already known since 1954.⁴ Many other examples can be found in the literature, where surfactant is brought into contact with water,⁵ or a neutralizing acidic aqueous phase.⁶ Also the process of soil removal has been studied by a contact experiment of a micellar solution with an oil/long chain alcohol phase.⁷ Unfortunately the molecules cannot be followed individually, but electron microscopy is as close as we can get to elucidate the mechanism underlying the process of rearrangement of molecules during the penetration experiment. Since this is a dynamic process, that cannot be studied as such in an electron microscope, the penetration process should be frozen in, capturing the transitions in action. In this study we describe a contact experiment on an electron microscopic scale. Use was made of the cryo-transmission electron microscope (cryo-TEM) technique, where very thin vitrified specimens were observed at low temperature in a, for this purpose, specially equipped electron microscope. There is a literature precedent for endeavors to catch dynamic processes in action by vitrification, and using the cryo-TEM technique.^{8,9}

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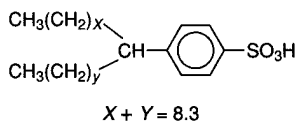
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Experimental Section

Materials. Commercial dodecylbenzenesulfonic acid (HDoBS, "MARLON AS3", Hüls, 98.3% pure, provided by Unilever Research Laboratory, Vlaardingen, The Netherlands) has an average composition as sketched, containing a few tenths of a percent sulfuric acid and the remaining part is non-surface-active organic material. It was used as received. Although the material is hygroscopic, the water content was still below the amount of water needed to induce a lyotropic lamellar phase, which is approximately 14 wt % water.¹⁰



Specimen Preparation. Satisfactory and reproducible results were obtained by the following procedure. Copper grids (400 mesh) were coated with a Formvar film; carbon was deposited on this film. One hour before use, the grids were glow-discharged in pentylamine vapor surroundings for 20 s. A minute amount of a 1 wt % solution of HDoBS in absolute methanol (p.a.) was nebulized onto the grid. The methanol was allowed to evaporate in a stove at 60 °C for 1 to 2 h. The grid was then mounted in a Reichart-Jung KF80 plunging device, minute amounts of water or a 1 *M* sodium hydroxide solution in water (typically less than 50 $\mu\text{L}/\text{cm}^2$) were nebulized onto the grid, and, as soon as manual handling allows (less than 3 s), the grid was guillotined into liquid ethane that was cooled to below -170 °C by liquid nitrogen. The grid with the vitrified material was transferred to the precooled specimen holder (Gatan cryo holder, model 626) in liquid nitrogen. The specimens were observed in a Philips CM 20 electron microscope operating at 200 kV with an anticontaminator device. The specimens were kept below -170 °C throughout the experiment and also in the microscope. The samples were surveyed at low magnification (3800 times). Selected areas were photographed twice at high magnification (44000 or 50000 times) using a low dose technique. The first exposure was taken at an underfocus value varying between 0.5 and 1 μm ; the second exposure was defocused 1 μm further. Images were recorded on Agfa 22D56 sheets, developed in Kodak D19 developer for 12 min.

Light Microscopy. Glass microscopy slides were treated as a closest possible mimic of the treatment of the electron microscopy grids. The slides were coated by covering with a solution of Formvar, the solvent (1,2 dichloroethane) was allowed to evaporate, carbon was deposited, and the slides were glow discharged in pentylamine vapor surroundings for 20 s. Surfactant (HDoBS) was nebulized as described above, the slides were dried at 60 °C for 2 h. After nebulizing of water or sodium hydroxide solution, the slides were observed (without coverslip) with a Zeiss Axiophot light microscope, equipped with an automatic photocamera, using the crossed polars and the phase contrast mode. Due to drying artifacts (especially for the NaOH case), the images needed to be photographed as soon as possible. In this short time it was impossible to obtain adequate photographs of the samples between crossed polars. Glass slides treated in this way have a hydrophilic surface; a droplet of water spreads out over the surface (a small contact angle, $<5^\circ$), whereas a slide with only a Formvar coating is much more hydrophobic (contact angle $>45^\circ$).

Results and Discussion

Dodecylbenzenesulfonic acid (HDoBS) is a viscous liquid at room temperature. This feature can be exploited for cryo-TEM studies, since only very thin specimens can be used with this technique. When subsequently the surfactant and the aqueous phase are nebulized onto the grid, thin specimens can be obtained, with several contact sites between the aqueous phase and a surfactant droplet, which can be studied after vitrification. However, at many

(9) Also the freeze fracture electron microscopy technique can be employed for contact experiments, which has the advantage of employing a slightly thicker sample. Sein, A.; Engberts, J. B. F. N. To be submitted for publication.

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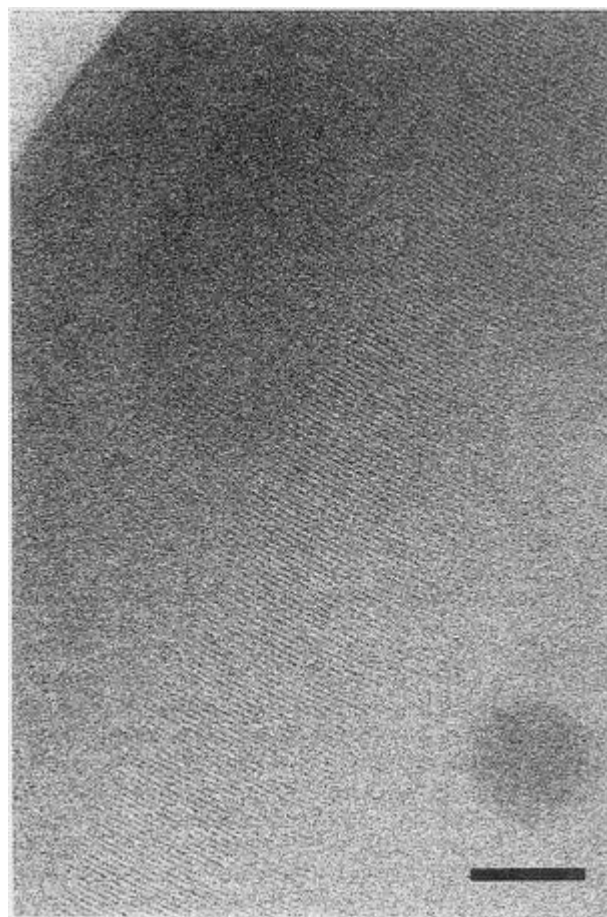


Figure 1. Image of untreated HDoBS collected in a fold of the support film, showing unusual order of surfactant molecules, with a periodicity of 2.7 nm, carried on over a distance of 1 μm . The image was obtained with the microscope set $\sim 0.5 \mu\text{m}$ under focus. The bar represents 50 nm.

contact sites the penetration of aqueous phase aligns the surfactant molecules in a direction that will not have any contrast with respect to the direction of the electron beam. The standard method would be blotting off a droplet of aqueous phase brought on a grid that was coated previously with a thin film of surfactant. Initially such experiments have been executed, however, they were without result. It is too destructive for the delicacy of the small scale penetration experiment, and furthermore, the process would take too long to be able to observe the phase transitions.

Surfactant before Contact with the Aqueous Phase. Tiny droplets of HDoBS were spread out over the grid, as well as accumulations of surfactant at the rims of a mesh, by nebulizing a solution of HDoBS in methanol on the grid and allowing the solvent to evaporate. Some mesh is fully covered with surfactant, too thick to observe any sort of penetration. In general these droplets and accumulations seem to be amorphous, and no order can be observed by cryo-TEM. When the HDoBS has collected in a fold in the Formvar-carbon film, extreme long-range order was detected in limited localized areas. A detail of such a fold filled with HDoBS is shown in Figure 1. The line pattern here continues over a distance of at least 1 μm in a direction perpendicular to the lines, the periodicity is 2.7 ± 0.2 nm.

Surfactant in Contact with Water. When the small congeries of amorphous surfactant described above are brought into contact with water, the surfactant melts off and is solubilized into the water phase. Water typically makes the HDoBS flow away as also can be observed by light microscopy (see below). This process, after vitrifica-

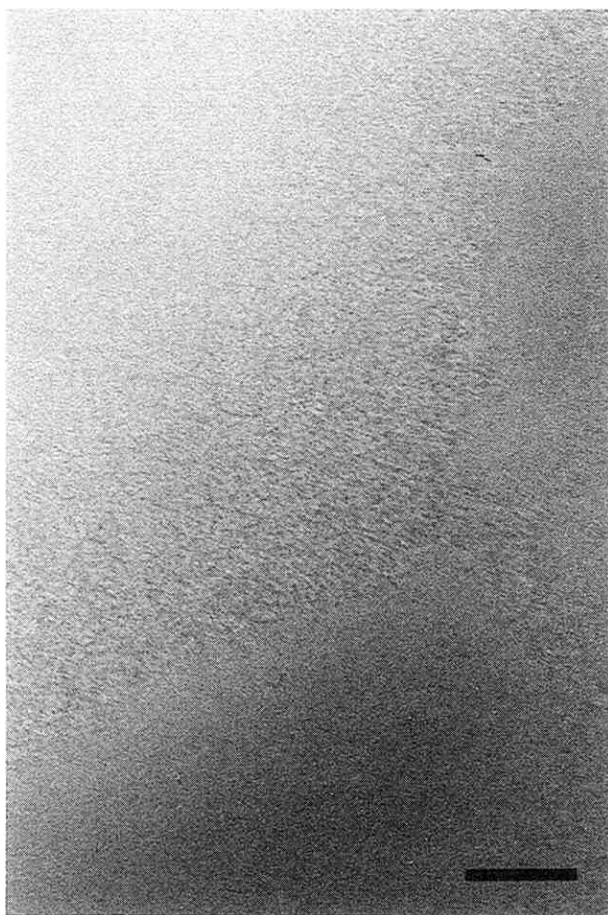


Figure 2. Contact of a rather thick bulk HDoBS phase with water, showing the melting off of the surfactant into smaller units. The image was obtained with the microscope set $\sim 1.5 \text{ mm}$ under focus. The bar represents 100 nm.

tion, is visualized in Figure 2. The dark lower right part of the micrograph is the surfactant phase; water comes from the upper left corner. The speckled middle area is where the bulk surfactant melts off into smaller units finally emerging as micelles, going toward the upper left corner. This is also the direction of transition, as can be inferred from the weak striated character, reminiscent of water withdrawing from a beach.

The water also penetrates into the bulk surfactant phase, inducing order of the surfactant molecules. When the specimen layer is thin enough, the order appears as structures that look like fingerprints. Some examples of these fingerprints are shown in Figure 3. Going toward the lower left corner of Figure 3A, more and more surfactant has accumulated; therefore the image becomes darker. The periodicity of this order of surfactant molecules is in all cases $2.9 \pm 0.2 \text{ nm}$. Comparison of this type of order with that of HDoBS that has not yet been contacted with water (Figure 1) shows that in the contact experiment water induces much more curvature and less long-range order.

Surfactant in Contact with Sodium Hydroxide Solution. The industrial surfactant is in its acidic form, HDoBS, and is used as such when it is processed for commercial applications. In these processes, the surfactant is neutralized by sodium hydroxide solution in water. In this way, the common sodium dodecylbenzenesulfonate surfactant (NaDoBS) is obtained.¹¹ Moreover, a sodium hydroxide solution is an electrolyte solution. Salts can induce and stabilize the lamellar arrangement of NaDoBS molecules in aqueous surroundings.^{2,3}

Contacting HDoBS with sodium hydroxide solution clearly gives different results from those with water. The alkaline solution induces order over a more extended region, and also distinct boundaries are observed between areas with bulk HDoBS and patches of alkaline solution.

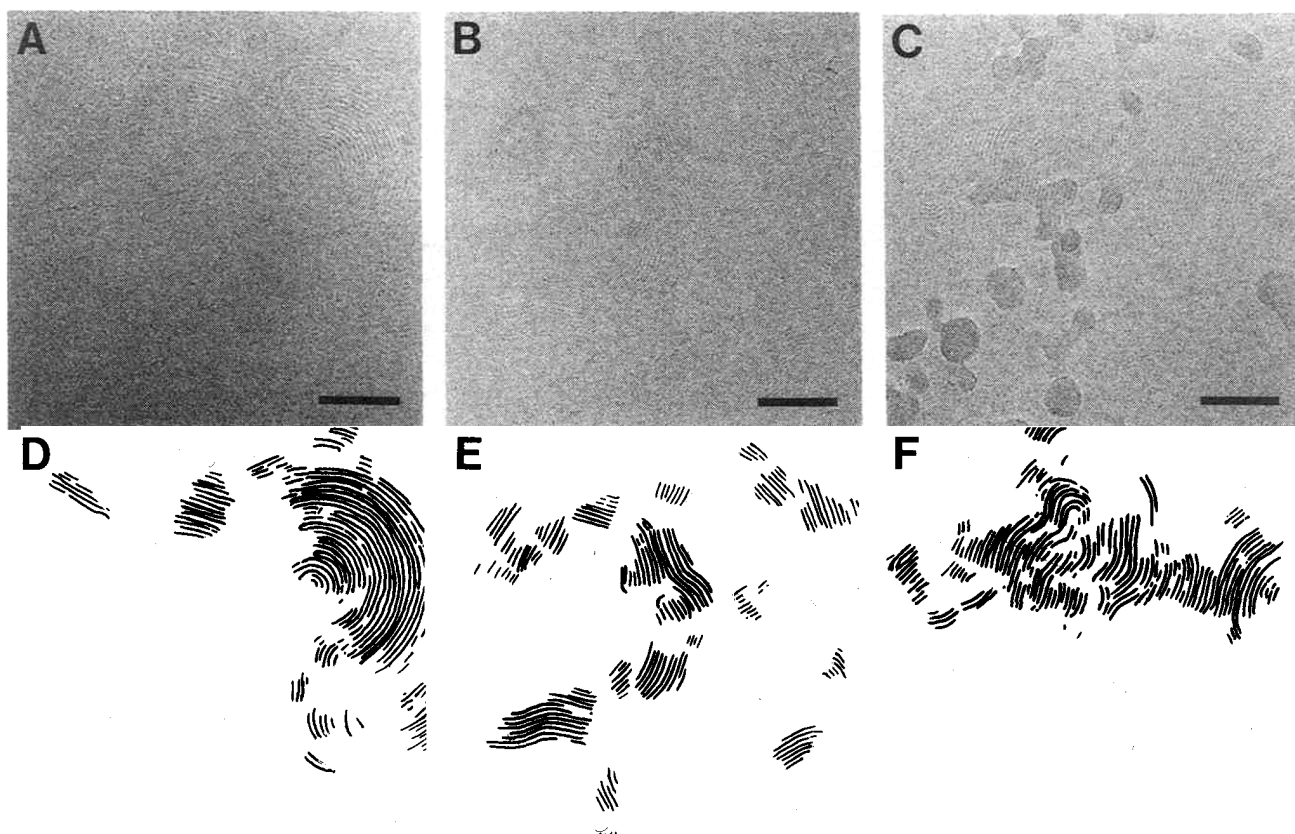


Figure 3. Contact of a fairly thin patch of HDoBS with water, showing the emergence of small fingerprint patterns, with a periodicity of 2.9 nm (A-C), and a schematic representation of the line patterns (D-F). The images were obtained with the microscope set $\sim 1.5 \text{ mm}$ (A), $\sim 0.5 \text{ mm}$ (B), and $\sim 0.8 \text{ mm}$ (C) under focus. The bars represent 50 nm.

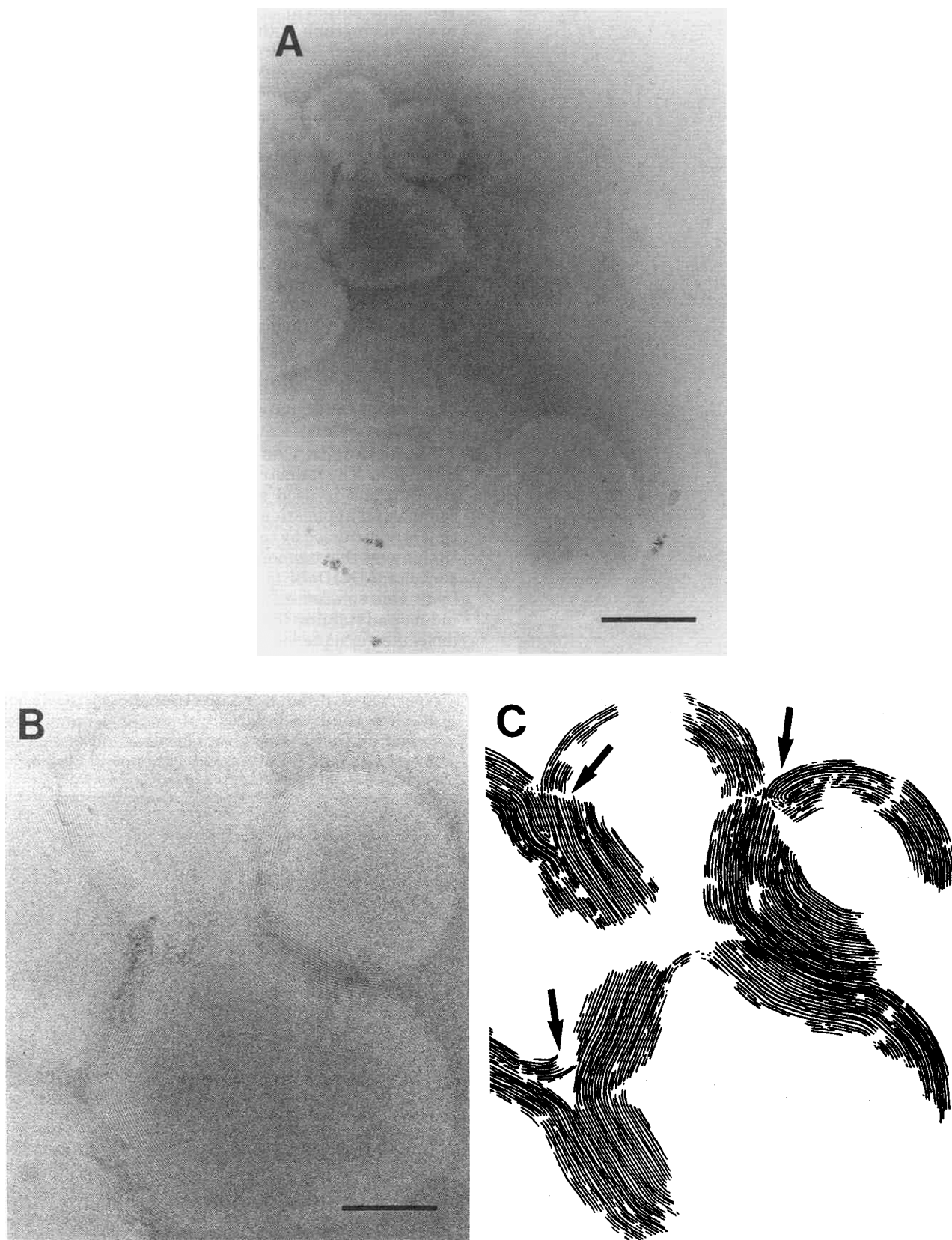


Figure 4. Contact of a fairly thin patch of HDoBS with a 1 *M* sodium hydroxide solution. (A) Survey showing the darker patch of HDoBS and the lighter spots of sodium hydroxide solution. The image was obtained with the microscope set $\sim 0.5 \mu\text{m}$ under focus. The bar represents 250 nm. (B) Photographical magnification of one of the contact sites, showing the extended fingerprint pattern, with a periodicity of 2.9 nm. The bar represents 100 nm. (C) Schematic representation of the pattern seen in (B). Notice the dislocations, marked by arrows.

A beautiful example of the order induced by a sodium hydroxide solution is shown in Figure 4. The survey (Figure 4A) shows the darker accumulation of amorphous HDoBS (as also was observed for the samples not contacted with water, see above) and the lighter drops of vitrified

sodium hydroxide solution. Figure 4B shows details at higher magnification. The order of surfactant molecules is clearly seen and is highlighted by a schematic repre-

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Figure 5. Penetration of a sodium hydroxide solution (right-hand side) into a rather thick HDoBS phase, showing small spots of molecular alignment with a periodicity of 3.7 nm. The image was obtained with the microscope set ~ 1.5 μm under focus. The bar represents 50 nm.

sensation in Figure 4C. Again, the periodicity in these fingerprints is 2.9 ± 0.2 nm. Notice also the several discontinuities in the fingerprint pattern, which result from dislocations in the arrangement of the surfactant molecules.

Not all contact sites yield these ordered structures over larger distances. More common is a boundary as shown in Figure 5. The alkaline solution (coming from the lower right) is still involved in the initial stages of penetration and only small domains of ordered molecules at the boundary between the bulk surfactant phase (upper left) and the sodium hydroxide solution are caught by vitrification. A vague striated appearance is present, as also can be seen in Figure 2, with a periodicity of approximately 3.7 ± 0.3 nm. Probably the specimen is too thick in this area to create fingerprints as shown in Figure 4. Another difference between Figures 4 and 5 is that in the latter, the orientation of the apparent order is perpendicular to the boundary between bulk surfactant phase and aqueous phase, whereas it is oriented parallel to the boundary in Figure 4. Moreover, it is remarkable that, generally, the sodium hydroxide solution (electrolyte solution) parts and, even more so, the border regions between alkaline solution and bulk surfactant phase are more sensitive to beam damage than areas with only surfactant or (pure) water. Since most images were photographed twice (at two defocus values), this damage is easily seen in a sequence of two photographs. This can be seen in Figure 5, which is the second exposure of the sequence. The right-hand side of the graph with sodium hydroxide solution and the border region are dearly damaged by beam irradiation, whereas the left-hand side with bulk surfactant seems rather unharmed.

Parallel Experiments by Light Microscopy. Obviously, contrastingly different images are obtained at contact sites of bulk surfactant with either pure water or sodium hydroxide solution. Looking for clues for the difference, the whole process was repeated by the use of a light microscope and with a light microscopy slide as a support instead of an electron microscopy grid. The slide was prepared in the same way as a grid for an electron microscopy experiment. Figure 6 shows some images of these treated glass slides, obtained by phase contrast light microscopy. After the HDoBS solution was nebulized and the solvent evaporated, droplets with diameters of 4–25 μm of bulk surfactant phase are spread over the slide. In Figure 6A, the typical congeries of HDoBS can be seen, often arranged in fairy circles. In some rare cases these droplets show some minute birefringence when observed

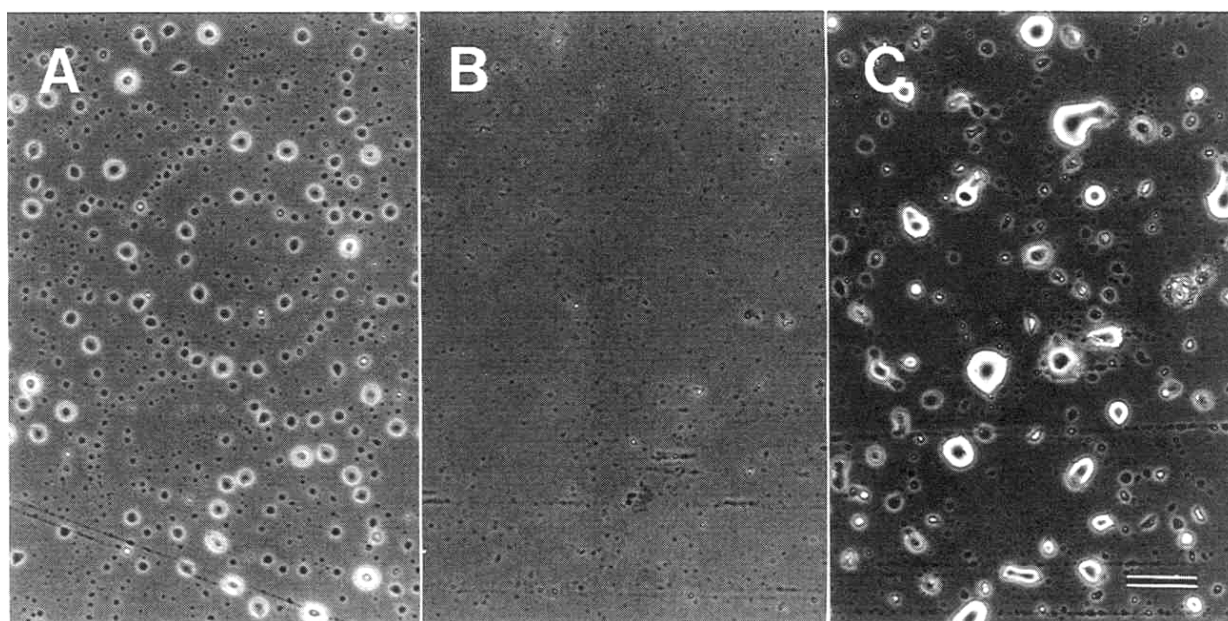


Figure 6. Phase contrast light microscopy images of light microscopy slides treated in a similar fashion as electron microscopy grids; (A) congeries of HDoBS, before contacting with an aqueous phase; (B) after nebulizing water, objects have faded away; (C) after nebulizing 1 M NaOH solution, odd-shaped islands of material. The bar represents 50 μm .

between crossed polars (not shown) and, if so, only on one side of the droplet. This is caused by some long-range order and is probably a small patch of lyotropic lamellar phase, which is due to locally high concentrations of water still present in the HDoBS or taken up from the atmosphere.

These droplet-shaped accumulations of surfactant were not expected. By the glow-discharge treatment of the formvar-carbon layer in pentylamine vapor surroundings (see Experimental Section) a rather hydrophilic surface of the substrate was obtained (as was also demonstrated by the spreading of a droplet of water over the surface, see Experimental Section). The surfactant solution would spread out completely as a homogeneous thin film over such a hydrophilic surface, remaining as such after evaporation of the solvent. A crucial point in this procedure is the process of spreading. Factors that determine the spreading are (i) the nature of the surfactant and its molecular structure, viscosity, and surface tension, (ii) the nature of the surfactant solution in methanol, in particular the surface tension, (iii) the nature of the surface over which the surfactant should spread, predominantly the surface tension, and (iv) the humidity of the surrounding air.¹²

The nebulizing of pure water only fades away the droplet character. When viewed by light microscopy, the flowing away induced by water seems to enhance the amorphous state of the surfactant phase. As is shown in Figure 6B, distinct structures are hardly observable by light microscopy.

Strikingly different is the image after nebulizing a sodium hydroxide solution. Now many odd-shaped pieces of material are distinctly observable by light microscopy (see Figure 6C). Even between crossed polars, birefringent objects are observed (not shown, see Experimental Section) before excessive drying ruins the sample. This is indicative for the long-range order induced by the sodium hydroxide solution. The difference between the behavior of pure water and that of sodium hydroxide solution can be traced back to the different lyotropic liquid-crystalline behavior of HDoBS as compared to NaDoBS (obtained after neutralization). Another factor is that the sodium hydroxide solution is an electrolyte solution, and it will not have all water molecules available to instantaneously hydrate the surfactant headgroup. On a larger scale, this difference does not seem to play a role in the first, lamellar-arrangement inducing water penetration.¹⁰

Fingerprints. The fingerprint patterns of thin dark lines are caused by a local high concentration of atoms with a relatively high atomic number: sulfur in the surfactant headgroup. Apparently the pattern only shows up in very thin sections of the specimen. The pattern must be induced by a particular arrangement of the surfactant molecules that originates from the presence of the aqueous phase. A possible explanation for the pattern comes from the dimensions of the surfactant molecule. The hydrophobic tail is calculated to be maximally 1.4 nm long for an all-trans chain in the dodecylbenzenesulfonate molecule, but a reasonable thickness for the hydrophobic part of the bilayer appears to be 2.0 nm.¹³ The observed periodicity of 2.9 nm is also comparable to the repeat distance of a lyotropic lamellar phase of NaDoBS in water and of HDoBS at the minimum amount of water to induce a lyotropic lamellar phase,¹⁰ and to literature values of several metal ion-dodecylbenzenesulfonate lyotropic lamellar systems.¹⁴ Most likely, a fingerprint pattern arises in a monolayer, or at most in several layers of

surfactant molecules, lying flat on the surface of the substrate. The molecules are aligned in rows, with the tails touching each other and the sulfonate headgroups pointed toward a small channel of aqueous phase, in a two-dimensional lamellar arrangement of amphiphilic molecules. Similar images were obtained by other techniques to visualize a lamellar phase by electron microscopy, such as cryo-microtomy¹⁵ or microtomy on amphiphilic systems in the lamellar phase that are fixed or polymerized¹⁶ and also on cast thin films of polymers with thermotropic liquid-crystalline properties.¹⁷ Negative staining of amphiphilic material capable of forming a lyotropic lamellar phase also can show fingerprint-like patterns.^{1c,16,18} However, whether these really represent the natural way of aggregation in aqueous media remains a point of discussion, since it has been proven that drying and staining artifacts also can induce images that look like the fingerprint patterns.¹⁹ Even if that would be an artifact, the molecular arrangement of amphiphiles on the electron microscopy grid will be as described above for the vitrified contact experiment. Both HDoBS and NaDoBS only form a lamellar phase as their only lyotropic liquid-crystalline phase¹⁰ and are not prone to form an inverted hexagonal (H_{II}) phase. It is therefore unlikely that the observed pattern can be explained by the presence of an inverted hexagonal arrangement of the surfactant molecules, despite the fact that the electron micrographs have a similar appearance as the micrographs from cryo-TEM of the H_{II} phase.^{8a,20} Another indication that this cannot be an H_{II} phase is that only one repeat distance is found (2.9 nm), whereas the H_{II} commonly shows two repeat distances, corresponding with 1:1 and 1: $\sqrt{3}$ spacings.

The Mechanism of Ordering. The molecules in the bulk surfactant droplet are expected to be in an amorphous, liquid-like arrangement. When this bulk surfactant is brought into contact with water, the bulk surfactant will be solubilized into the water or broken apart into smaller units, eventually emerging as micelles (Figure 2). This probably occurs when a water droplet hits a fairly thick congener of bulk surfactant. The penetrating water molecules align the surfactant molecules through directional bonding, all heads pointing to the water, the tails turning away. Although contact between water and HDoBS must have been made, the images appear optically isotropic. Particularly when the surfactant layer on the substrate is several molecular layers thick, and shows no observable structure, we suggest that on most places the surfactant molecules are aligned by the penetrating aqueous phase more or less perpendicular to the surface of the Formvar-carbon substrate.

On the other hand, when water hits the surfactant layer, it tends to spread out as a thin film over the surfactant layer. When the surfactant layer is thin enough, the water penetration might induce the observed fingerprints. An "alignment nucleus", that can be a coincidental local order of surfactant molecules, accepts the water to hydrate the headgroups. Henceforth, the ordering of surfactant molecules is carried on, creating a kind of water channel,

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that receives the water molecules from the water film spread out over the surfactant layer. However, in the case of only water, this channel will extend over a rather small distance. Once a channel has been formed, it can act as a template for the next one, and the parallel lines of the fingerprint pattern emerge, as exemplified in Figure 3.

The contact of bulk surfactant with sodium hydroxide solution also produces two types of patterns. For a relatively thin layer of surfactant, again fingerprint patterns have been observed (Figure 4). For thicker patches of surfactant, the alignment of molecules is less clear (Figure 5). When a microdroplet of sodium hydroxide solution hits a thin layer of surfactant, it initially spreads out over the surfactant layer. An alignment nucleus (see above) accepts the water for hydration and, more importantly, the hydroxide ions to neutralize the acidic headgroups. This induces the growth of molecular alignments by accepting aqueous phase from the thin film of sodium hydroxide solution, which has been spread out over the surfactant phase, rather than by allowing the aqueous phase to penetrate through the channels. Again, a penetration channel can form, which acts as a template for the formation and the growth of a next one, parallel with the first one, and so on.

Presumably, the dislocations (Figure 4) arise either from local disorder or perturbations in the surfactant layer that are encountered by the growing channel or from a mismatch of growing channels that encounter each other.

The contact of a thicker accumulation of surfactant with sodium hydroxide solution presumably results in mutual penetration. Again, most of the sodium hydroxide droplet will spread out over the surfactant layer before it penetrates into the surfactant phase. The striated appearance, oriented roughly perpendicularly to the outer penetration front, is only left as a witness of the penetration process. Just on this penetration front, the alignment of surfactant molecules by the penetrating sodium hydroxide solution can be seen locally (Figure 5). The typical repeat distance is significantly larger (ca 3.7 ± 0.3 nm) than for the cases with a regular fingerprint pattern. This cannot be due to swelling of the interbilayer water layer, since NaDoBS does not swell in excess water.¹⁰

Conclusions

Technique. Contact or phase-penetration experiments can be successfully conducted using a cryo-transmission electron microscope. This yields information almost on the molecular level on how interpenetrating phases induce molecular rearrangements. We contend that many other systems can be studied fruitfully in a similar fashion, as long as only tiny accumulations (or microcrystals) of surfactant can be spread out over the grid. Currently, endeavors are undertaken to study the process of fusion of vesicles of synthetic amphiphiles, induced by Ca^{2+} , in a similar fashion. Unfortunately, the phase transitions

in the thin and small patches occur too fast to elucidate them clearly and mechanistically. This problem might be overcome by plunging the grid covered with the tiny amounts of bulk surfactant through a thin cloud of nebulized aqueous phase into the vitrifying agent.²¹ By such a procedure, the time between contact of the aqueous phase with the bulk surfactant and the vitrification can be varied. This is also the time that the penetration process can occur, and variation of this time interval is an important factor in the study of the transformation mechanism. A disadvantage is that penetration can induce alignment of surfactant molecules that still appears isotropic in the electron microscope. This disadvantage can be minimized by nebulizing both phases onto the grid. Also note that such a small scale contact experiment might not be representative for the situation in the bulk phase.

Contact with HDoBS. The cryo-TEM phase penetration experiment has been applied to the study of the alignment of HDoBS molecules through contact with water or a sodium hydroxide solution. Most remarkable is the appearance of fingerprint patterns. These emerge from alignment nuclei, that facilitate the first penetration, followed by hydration and neutralization of the headgroups. The periodicity and dimensions of the pattern imply a molecular alignment as a two-dimensional lamellar phase. This type of pattern only grows in fairly thin layers of surfactant. Obviously, contact with a neutralizing alkaline solution has much more impact on the alignment process than pure water has. Thicker contact sites yield less clear molecular alignment, only over limited distances.

It has been noted by van der Linden²² that the here described fingerprint patterns bear resemblance with the networks of graphite after electron beam irradiation.²³ The rearrangement of surfactant molecules from amorphous to aligned must involve a similar process as the rearrangement of carbon atoms from graphitic soot to spherical particles and nanotubules.

The contact experiment employing cryo-TEM is a promising technique to study the dynamics of transitions in lyotropic liquid-crystalline behavior of amphiphilic molecules with water.

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